THE CARCINOGEN ASSESSEMENT GROUP

PRELIMINARY

REVIEW OF ONCOGENICITY

OF

~~AVADEX~~
(Diallate, Triallate)

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AVADEX

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I. CONCLUSIONS

A statistically significant increase in tumor incidence has been demonstrated for Avadex (diallate) but not for triallate. The evidence for diallate involves four independent studies in rats and mice.

In mice, the Innes study showed statistically significant and pronounced increases in hepatoma incidence in males in the two tested strains. There were small but statistically significant increases in the incidence of lung adenomas in both sexes in one strain (X) of mice and in males only of the other strain (Y). The Boots study on female mice showed no statistically significant excess incidence of tumors, in each individual organ site.

Regarding the effects of Avadex (diallate) on rats, the Litton Bionetics study showed a statistically significant increase in malignant tumors, as a whole, only at the highest dose in male rats and only at the lower dose in female rats. The Industrial Bio-Test study in rats showed a statistically significant excess of mammary carcinomas in females.

Concerning triallate, the Industrial Bio-Test study in rats is the only available study for this compound. It did not show a statistically significant increase in tumor incidence.

II. METABOLISM AND MUTAGENESIS

A) METABOLISM STUDY

A study of the metabolic fate of radioactively labeled Avadex injected intraperitoneally in rats revealed that, with 88% of the administered compound radioactively accounted for in 48 hours after injection, 61% appeared in urine, 19% in feces and 8% as exhaled CO2.(1) The urinary metabolites were not identified.

B) Mutagenesis

Dominant lethal tests of Avadex (diallate) and Avadex BW (triallate) in albino Charles River mice were performed by Industrial Bio-Test Laboratories. (2) Each compound was injected intraperitioneally with corn oil into groups of male mice. Each of the two studies was performed with a negative control group using Metepo (tris-1-(2-methyl)-azidinyl phosphine oxide). After treatment, the males were mated sequentially with untreated females during a six week period and the females were examined in an early stage of pregnancy for the number of implanted embryos and number of embryonic deaths. Avadex was treated at doses of 100 and 200 mg/kg body weight, and avadex BW at 200 and 400 mg/kg body weight.

The results of these tests were that treated groups did not show any significant decrease in the number of viable offspring compared with the negative control groups whereas the positive control groups did show a significant reduction in the number of viable offspring, as expected. Therefore, the compounds do not cause dominant lethal mutations in treated males under the conditions of the test.

Mutagenesis tests were performed by Andersen, et al. (10) on a series of 110 herbicides including diallate and triallate in Salmonella typhimurium and on 35 herbicides including triallate in a T4 bacteriophage/E. coli test system. Neither system included metabolic activation and the concentrations were not stated. None of the 110 compounds induced a significant number of mutations in the Salmonella tests, and only 4 compounds, not including triallate, were marginally significant in the T4/E. coli test. Although these experiments were negative, the inclusion of a metabolic activation system, which is the current accepted practice might produce a positive result. Therefore, the negative result must be considered tentative.

III. MOUSE STUDIES

A. Innes Study (Oral)

The maximal tolerated dose of Avadex was given to two hybrid strains of mice, (C57BL/6 x C3HAnf) F1 designated as "strain X" and (C57Bl/6 x AKR)F designated as "strain Y" mice.(3) There were 18 treated mice and 18 untreated controls of each strain and each sex. Two hundred fifteen mg/kg body weight was given in gelatin daily by stomach tube beginning when the mice were 7 days of age. After the mice were weaned at 28 days of age, 560 ppm Avadex was mixed directly in the diet and provided ad libitum. Treatment was continued for 84-86 weeks. Pooled controls represent four different untreated control groups, each in a separate room and an additional control group given gelatin suspension.

Postmortem observations included thorough external examination and examination of the thoracic and abdominal cavities, with histologic examination of major organs and of all grossly visible lesions. The cranium and thyroid glands were not dissected. Blood smears were examined on all mice with splenomegaly of lymphadenopathy.

There were significantly elevated incidences in hepatomas of the liver in males of the X strain ($p=<1.57x10^{-5}$) and males of the Y strain ($p=<3.8x10^{-3}$), when compared to the matched control mice. Metastases were not observed; however, in this particular study, the mice were killed after 18 months, rather than allowing the mice to survive 2 years or more, a time long enough for metastases to have probably occurred.

NUMBERS OF MALE AND FEMALE MICE
INGESTING AVADEX WITH TUMORS OF THE LIVER

Strain	Dose (ppm)	Tumors of the Liver	
		Male	Female
X Matched	0	0/16(0%)	0/16(0%)
X Pooled	0	8/79(10%)	0/87(0%)
X	560	13/16(81%)	3/16(19%)
Y Matched	0	1/18(6%)	0/17(0%)
Y Pooled	0	5/90(6%)	1/82(1%)

Tumors of the lung were slightly increased in strain X male mice (4 of 16 = 25%), compared with 0 of 16 control matched male mice (0%) and 5 of 79 (6%) pooled control male mice ($p<0.05$). Six of 32 strain X male and female mice (19%) and 0 of 32 control male and female mice (0%) developed lung tumors.

NUMBERS OF MALE AND FEMALE MICE INGESTING AVADEX WITH TUMORS OF THE LUNG

Strain	Dose (ppm)	Tumors of the Lung	
		Male	Female
X Matched	0	0/16(0%)	0/16(0%)
X Pooled	0	5/79(6%)	3/87(3%)
X	560	4/16(25%)	2/16(13%)
Y Matched	0	2/18(11%)	1/17(6%)
Y Pooled	0	10/90(11%)	3/82(3%)
Y	560	4/18(22%)	1/15(7%)

There were increases in numbers of animals with tumors in one or more organs. Tumors other than liver and lung generally were lymphomas.

Fourteen of 16 (88%) strain X males ($p < 10^{-9}$), 12 of 18 (67%) strain Y males ($p < 0.00684$), and 5 of 16 (31%) strain X female mice (p 0.0515) had tumors, when compared to matched control mice.

NUMBERS OF MALE AND FEMALE MICE INGESTING AVADEX WITH TUMORS IN ANY ORGAN

Strain	Dose (ppm)	Tumors in Any Organ	
		Male	Female
X Matched	0	0/16(0%)	0/16(0%)
X Pooled	0	22/79(27%)	8/87(9%)
X	560	14/16(88%)	5/16(31%)
Y Matched	0	3/18(17%)	2/17(12%)
Y Pooled	0	16/90(18%)	7/82(8%)
Y	560	12/18(67%)	2/15(13%)

In conclusion, Avadex is carcinogenic for liver and one or more organs in strains X and Y male mice, for one or more organs in strain X female and for lung in strain X male mice.

B. Intraperitoneal Carcinogenicity Study in Mice (Boots)

Female albino mice (obtained from Schofiel), weighing 18-29 grams, were given weekly intraperitoneal injections of 5 mg. of the active ingredient of Avadex, CP-15336 (2% suspended in 10% gum acacia) for 10 weeks.(4) There were 50 mice per group. Controls received the vehicle alone. Mice ingested Oxoid modified diet 41B for the first 4 months and Oxoid breeding diet for the remainder of the study. Twenty mice were killed at the end of 37 weeks and the remainder after 72 weeks.

Necropsies were done on mice that died or were killed. Superficial glands, liver, spleen, kidney, adrenal, stomach, urinary bladder, thymus and skin were examined grossly. Abnormal tissues were examined histologically. Histologic sections of lungs were routinely performed.

Mice given intraperitoneal injections of the active ingredient of Avadex developed more carcinomas of the mammary gland as the control mice but the difference isnot statistically significant. There were metastases from the mammary gland to the lung in one mouse.

TUMORS OF THE UTERUS AND MAMMARY GLAND IN FEMALE MICE GIVEN AVADEX INTRAPERITONEALLY a)

Group	No. Mice with Mammary Gland Tumors and Uterine Tumors
Treated	6/25(24%)
Control	3/29(10%)

a) the active ingredient of Avadex

Three treated mice had tumors of the liver, compared to one of the control mice. One treated mouse had anaplastic adenocarcinoma of the gland adjacent to the eye with metastases in the lungs.

In summary, the active ingredient of Avadex given intraperitoneally increased the incidence of tumors of the mammary gland and uterus, but not significantly. The tumors were more malignant in the treated mice. There also were more tumors of the liver in treated mice.

C. Dermal Carcinogenesis Study in Mice (Boots)

Female albino mice (obtained from Schofiel) weighing 18-29 grams, were given weekly skin applications of 75 mg. of the active ingredient of Avadex, CP-15336 (30% in acetone) for up to 76 weeks and killed. 1) There were 50 mice per group. Controls were the mice from a lung tumor study that received intraperitoneal injections of gum acacia.(3)2) Mice ingested Oxoid modified diet 41B for the first 4 months and Oxoid breeding diet for the remainder of the study.

The hair of the back, from the scapula to the tail, was clipped before treatment and at intervals whenever necessary. The chemical solutions, calibrated by pipette at 0.25 ml per application, were spread as evenly as possible over the clipped surface.

Necropsies were done on mice that died or were killed. Superficial glands, liver, spleen, kidney, adrenals, stomach, urinary bladder, thymus and skin were examined grossly. Abnormal tissues were examined histologically. Histologic sections of lungs were routinely performed.

Female mice receiving dermal applications of the active ingredient of avadex developed more than 2 times as many tumors of the uterus and mammary gland than did mice given gum acacia ($p=>.05$). One of the avadex-treated mice had carcinoma of the mammary gland with multiple metastases to the lungs, and another had an undifferentiated sarcoma in the uterus, indicating that the tumors were more malignant in the mice receiving avadex than in the controls.

TUMORS OF THE UTERUS AND MAMMARY GLAND
IN FEMALE MICE GIVEN DERMAL APPLICATIONS OF AVADEX a)

Group	No. Mice with Mammary Gland and Uterine Tumors
Treated	10/46(22%)
Control b)c)	3/29(10%)

a) The active ingredient of avadex, dissolved in acetone.
b) Group of control mice for a long-term study that had received gum acacia intraperitoneally for 10 weeks. (3)
c) Thirteen mice were unaccounted for in the data.

1) Avadex consists of 41.99% CP15336, 54.03% Panola heavy aromatic naphtha, and 4.00% of emcol, AD4-20.
2) Twenty were killed at the end of 37 weeks, and are not included in these results.

Five of 46 female mice (11%) given Avadex developed tumors of the liver and another a hemangioma of the liver compared to 1 control mouse with a liver tumor. Another Avadex-treated mouse had osteogenic sarcoma with metastases in multiple organs.

In summary, the active ingredient of Avadex applied dermally increased slightly, but not significantly, the incidence of tumors of the uterus and mammary gland. Those tumors were more malignant in the treated mice. Treated mice also had more tumors of the liver.

D. Skin Tumor-Initiating Study in Mice (Boots)

Female albino mice (obtained from Schofiel) weighing between 18-29 grams, were given weekly skin applications of 75 mg. of the active ingredient of Avadex, CP15336, (30% in acetone) for 10 weeks.(5) Another group of mice received weekly gastric intubation 15 mg CP15336 (6% in 10% gum acacia) for 10 weeks. The mice then received weekly skin applications of 0.25% croton oil for 25 weeks, and were killed at 37 weeks after the study began. The negative control was given acetone and the positive control consisted of urethane followed by croton oil. There were 30 mice in each group.

During the study "characteristic signs of CP15336 toxicity were seen in two mice treated with skin applications and in six mice dosed orally, . . ."

There were no significant differences in the numbers of mice with papillomas of the skin or forestomach between the controls and avadex-treated mice.

It was concluded that Avadex was not a tumor-initiating chemical when applied to the skin or forestomach under the conditions of this study.

E. Skin Tumor-Promoting Study in Mice (Boots)

Female albino mice (obtained from Schofiel) weighing 18-29 grams were given a single skin application of 150 micro-gram dimethylbenzanthracene (DMBA) in 0.1 ml. acetone. (5) Three weeks later 20 applications of 75 mg. avadex (30% in 0.25 ml acetone) were made at weekly intervals. A second group of mice received DMBA followed by acetone, and a third group received DMBA followed by croton oil. There were 20 mice in each group. Mice were killed 1 week after treatment was completed.

Skin tumors were not observed in mice painted with Avadex or acetone. There was a high incidence of skin tumors in the positive control mice.

It was concluded that avadex was not a skin tumor-promoting chemical when applied to the skin under the conditions of this study.

F. Innes Mouse Study (Subcutaneous)

Two hybrid strains of mice, (C57BL/6 x C3HAnf)F1 designated as "strain A" and (C57BL/6xAKR)F1 designated as "strain B" mice were used (5). Male and female mice, 18 per group, were given a single subcutaneous injection of 1000 mg/kg of body weight Avadex in corn oil on the 28th day of life and killed after 80 weeks. Control mice received corn oil only. The diet consisted of laboratory chow.

Tumors were not increased in mice given a single subcutaneous injection of Avadex.

IV. RAT STUDIES

A. Litton Bionetics Study

Avadex was administered in the diet to 26 male and 26 female Sprague-Dawley rats for 18 months and killed after 24 months. (7) The treated rats, divided by sex, were also divided into dosage groups, totaling 4 treated groups. The high dose, 300 ppm, was intended to be the maximal amount that the rats could tolerate for that period of time. The low dose was one-half the high dose. Two groups of 32 males and two groups 32 females received no treatment. Necropsies and complete histopathologic examinations were performed on all rats.

Average survival times for the male rats ingesting the high dose of Avadex were less than male rats in the other groups. One female rat given the high dose was killed at 28 weeks because of carcinoma of the mammary gland.

SURVIVAL TIMES (WEEKS) FOR MALES AND FEMALES INGESTING AVADEX

Dose	Females Average (range)	Males Average (range)
0	82.3 (44-93)	97.7 (63-104)
0	103.0 (94-104)	99.0 (45-104)
Low Dose	90.3 (47-104)	96.7 (57-104)
High Dose	91.3 (28-104)	83.5 (54-104)

Treated male rats developed significantly more malignant tumors than did control males (18/52 vs. 11/64) ($p = < .026$, 1-tail). This increase in malignancies was due to the increase in carcinomas in treated animals. There is a dose-related increase in the incidence of carcinomas in males where both the arithmetic dose response and the log (dose +1)-response relationship showed a significant increasing linear trend ($p < .02$).

MALIGNANT TUMORS IN MALE RATS INGESTING AVADEX

Dose	Incidence of Carcinomas	Incidence of Sarcomas	Incidence of Malignant Tumors a) b)
0	3/32 (9%)	1/32 (3%)	4/32 (13%)
0	2/32 (6%)	5/32 (16%)	7/32 (22%)
Low Dose	5/26 (19%) c)	1/26 (4%)	6/26 (23%)
High Dose	7/26 (27%)	5/26 (19%)	12/26 (46%)

a) Rats with carcinomas did not have sarcomas.
b) Corrected for survival.
c) Two rats with carcinomas had metastases (carcinoma of the prostate to lung and lymph nodes; islet cell carcinoma of the pancreas with metastases to the heart).

MALIGNANT TUMORS IN MALE RATS INGESTING AVADEX

Dose	Incidence of Carcinomas	Incidence of Sarcomas	Incidence of Malignant Tumors
0	5/64 (8%)	6/64 (9%)	11/64 (17%)
High Dose	7/26 (27%)	5/26 (19%)	12/26 (46%)

Female rats ingesting the low dose of Avadex had an increased incidence of malignant tumors, i.e. 11 of 26 (42%) vs. 8 of 64 (13%) ($p = < 0.005$).

The total number of rats with malignant tumors was statistcally significant ($p = < .0096$).

MALIGNANT TUMORS IN FEMALE RATS INGESTING AVADEX

Dose	No. Rats with Carcinomas	No. Rats with Sarcomas	Total No. Rats with Malignant Tumors a)
0	3/32 (9%)	2/32 (6%)	5/32 (16%)
0	1/32 (3%)	2/32 (6%)	3/32 (9%)
Low Dose	4/26 (15%) [b]	7/26 (27%)	11/26 (42%)
High Dose	1/26 (4%)	0/26 (0%)	1/26 (4%)

a) Rats with sarcomas did not have carcinomas.
b) One rat with carcinoma of the mammary gland had metatases to the lungs.

MALIGNANT TUMORS IN FEMALE RATS INGESTING AVADEX

Dose	No. Rats with Carcinomas	No. Rats with Sarcomas	Total No. Rats with Malignant Tumors
0	4/64 (6%)	4/64 (6%)	8/64 (13%)
Low Dose	4/26 (15%)	7/26 (27%)	11/26 (42%)

Both the number of rats with sarcomas and the total number of rats with malignant tumors were statistically significant, i.e., $p=<.01$ and $p=<.0014$ respectively.

Both male and female rats generally had reticulum cell sarcomas or leukemia. Male rats developed carcinoma of the prostate, malignant gliomas of the brain, and squamous cell carcinomas. Female rats had squamous cell carcinomas of the skin and lung and carcinomas of the mammary gland.

Three low dose female rats died with stomach hemorrhage and 2 with necrotizing inflammation of the stomach, and 3 others had focal hepatic necrosis. Diffuse necrosis of the liver was present in 3 females, focal hepatic necrosis in 1 female, and necrotizing inflammation in 1 female rat given the high dose of Avadex.

In summary, male rats ingesting the high dose of Avadex developed a significantly increased incidence of carcinomas, as well as malignant tumors. However, the incidence of sarcomas alone was not statistically significant. In female rats given the low dose of Avadex, the incidence of malignant tumors and sarcomas was significantly increased.

B. Industrial Bio-Test Study (Avadex BW)

A chronic oral toxicity study was carried out with Charles River albino rats. 3),1) Male and female rats, 50 in each group, ingested 0, 50, 100 or 200 ppm of Avadex BW Technical in dry, pulverized Purina rat chow for 24 1/2 months. 2), 3) Control rats were the same as for the Avadex study. Rats were housed individually in metal cages. Food and water were administered ad libitum.

Food consumption and body weights were done, as well as hematology, selected blood chemistries and urinalyses at periodic intervals were recorded.

Complete gross necropsies were carried out on all rats found dead, except for advance autolysis, and on all rats killed. Terminal weights were taken of brain, gonads, heart, kidney, liver and spleen. Detailed histophathological examinations were done on "selected animals sacrificed." All neoplasms and tissues with suspected "neoplastic lesions" were studied.

Male rats ingesting 200 ppm Avadex BW failed to gain weight during the first 3 weeks as did rats in the other groups ($p < 0.01$). Food consumption measurements disclosed that the amount of food eaten by test animals was similar to that of the controls.

Mortality increased after one year and it was somewhat higher in Avadex treated male rats.

MORTALITY (IN WEEKS) FOR MALE RATS INGESTING AVADEX - BW a/

Dose (ppm)	1-51	52-64	65-77	78-90	91-105	% Dead
0	2/50(4%)	3/50(6%)	10/50(20%)	7/50(14%)	13/50(26%)	34/50(68%
50	0/50(0%)	4/50(8%)	16/50(32%)	10/50(20%)	11/50(22%)	41/50(82%
100	3/50(6%)	7/50(14%)	7/50(14%)	8/50(16%)	10/50(20%)	35/50(70%
200	6/50(12%)	3/50(6%)	10/50(20%)	5/50(10%)	9/50(18%)	33/50(66%

a/ Number of rats dying at this time.

1) Charles River Breeding Laboratories, Inc., Wilmington, Mass.
2) CP 23436, Lot Va XHE-51, 95.3
3) Ralston-Purina Co., St. Louis, Mo.

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MORTALITY (IN WEEKS) FOR FEMALES INGESTING AVADEX - BW a/

Dose (ppm)	1-51	52-64	65-77	78-90	91-105	% Dead
0	1/50(2%)	0/50(0%)	6/50(12%)	9/50(18%)	12/50(24%)	28/50(56%)
50	2/50(4%)	2/50(4%)	9/50(18%)	5/50(10%)	11/50(22%)	29/50(58%)
100	4/50(8%)	2/50(4%)	7/50(14%)	11/50(22%)	11/50(22%)	35/50(70%)
200	1/50(2%)	2/50(4%)	4/50(8%)	12/50(24%)	13/50(26%)	32/50(64%)

a/ Number of rats dying at that time.

Leukocyte counts were depressed in male rats ($p < 0.01$) and female rats ($p < 0.05$) ingesting the higher dose for 12 months or longer. There also were significant decreases in hemoglobin in the females from the third to the 12th month.

Significant decreases in the weight of spleens were observed in females ingesting the chemical.

There were slight increases in benign and malignant tumors, other than the commonly occurring endocrine tumors, in rats ingesting Avadex. There were so many tumors in the untreated control rats, particularly in the females that results are difficult to interpret.

BENIGN AND MALIGNANT TUMORS IN MALE RATS INGESTING AVADEX - BW a/ b/

Dose (ppm)	Incidence of Benign Tumors	Incidence of Malignant Tumors	Incidence of Tumors
0	2/46 (4%)	4/46 (9%)	5/46 (11%)
50	0/48 (0%)	4/48 (8%)	4/48 (8%)
100	7/44 (16%)	2/44 (5%)	9/44 (20%)
200	4/41 (10%)	2/41 (5%)	5/41 (12%)

a/ Number of rats with endocrine tumors are not included.
b/ Some rats had benign and malignant tumors. Those rats are counted once.

The incidence of mammary gland tumors was about the same in control and treated female rats; however, there were more carcinomas of the mammary gland in rats ingesting avadex. Nine of 46 female rats (20%) given 100 ppm avadex had such carcinomas, compared to 4 of 48 control female rats (8%) ($p < .1$). It is likely that some, if not all, of the fibrosarcomas of the dermis were tumors of the mammary gland. These tumors would have further increased malignant tumors in that organ.

Pituitary adenomas were observed in similar numbers of treated and untreated rats. Some of these tumors were probably carcinomas in treated rats, as was found with tumors of the mammary gland. The tumors of the pituitary gland will be examined by CAG.

BENIGN AND MALIGNANT TUMORS OF THE MAMMARY GLAND IN FEMALES INGESTING AVADEX - BW

Dose (ppm)	No. Rats with Malignant Tumors	No. Rats with Benign Tumors	Total Number of Rats with Tumors
0	15/48 (31%)	4/48 (8%)	19/48 (39%)
50	12/48 (25%)	8/48 (17%)	20/48 (41%)
100	13/46 (28%)	9/46 (20%)	22/46 (48%)
200	13/45 (29%)	2/45 (4%)	15/45 (33%)

In this study, female rats ingesting 100 ppm Avadex had more carcinomas of the mammary gland than did the controls. Otherwise, the high incidence of tumors that was seen in control rats make interpretation of the results difficult. Histological examination of tissues in this study was limited.

C. Industrial Bio-Test Study

A chronic oral toxicity study was carried out with Charles River albino rats.(9)4) Male and female rats, 50 in each group ingested 0, 50, 100 or 200 ppm of Avadex Technical in dry, pulverized Purina rat chow for 24 1/2 months.5)6) Control rats were the same as for the Avadex BW study. Rats were housed individually in metal cages. Food and water were administered as ad libitum.

Food consumption and body weights were done, as well as hematology, selected blood chemistries and urinalyses at periodic intervals were recorded.

Complete gross necropsies were carried out on all rats found dead except for advanced autolysis, and on all rats killed. Terminal weights were taken of brain, gonads, heart, kidney,

4. Charles River Breeding Laboratories, Inc., Wilmington, Mass.
5. CP 23436, Lot Va. XHE-51,95.3
6. Ralston-Purina Co., St. Louis, Mo.

liver and spleen. Detailed histopathological examinations were done on "selected animals found dead, and on all remaining animals at the 24 month sacrifice." All neoplasms and tissues with suspected neoplastic lesions were studied.

Body weight gains were slightly less for female and male rats ($p = < 0.05$) ingesting 200 ppm Avadex than the control rats up to the 10th week. Food consumption of treated rats was similar to that of the untreated rats.

Mortality was somewhat increased in treated rats at the higher doses of Avadex.

MORTALITY (WEEKS) FOR MALE RATS INGESTING AVADEX a/

Dose (ppm)	1-51	52-64	65-77	78-90	91-105	% Dead
0	1/50(2%)	3/50(6%)	10/50(20%)	7/50(14%)	13/50(26%)	34/50(68%)
50	1/50(2%)	5/50(22%)	11/50(22%)	11/50(22%)	13/50(26%)	41/50(82%)
100	5/50(8%)	2/50(10%)	5/50(10%)	13/50(26%)	10/50(20%)	34/50(68%)
200	2/50(4%)	3/50(6%)	7/50(14%)	10/50(20%)	15/50(30%)	37/50(74%)

a/ Number of rats dying in the stated interval.

MORTALITY (WEEKS) FOR FEMALE RATS INGESTING AVADEX a/

Dose (ppm)	1-51	52-64	65-77	78-90	91-105	% Dead
0	1/50(2%)	0/50(0%)	6/50(12%)	9/50(18%)	12/50(24%)	24/50(48%)
50	0/50(0%)	2/50(4%)	3/50(6%)	8/50(16%)	17/50(34%)	30/50(60%)
100	2/50(4%)	1/50(2%)	4/50(8%)	10/50(20%)	15/50(30%)	32/50(64%)
200	3/50(6%)	1/50(2%)	10/50(20%)	7/50(14%)	17/50(34%)	38/50(76%)

a/ Number of rats dying in the stated interval.

Total leukocyte count was decreased significantly ($p = < 0.05$) in male rats ingesting 200 ppm Avadex between 6 and 24 months. The hemoglobin was significantly decreased in female rats ingesting 200 ppm between 3 and 12 months.

The weights of the spleen were significantly decreased in female rats ingesting 50, 100 or 200 ppm Avadex ($p = < 0.05$).

There were benign and malignant tumors (other than the endocrine tumors, which were commonly found in this strain) in 10 of 50 (20%) male rats ingesting 200 ppm of Avadex, compared to 5 of 50 control male rats (10%) ($p < .1$). Male rats ingesting 50 of 200 ppm avadex had more malignant tumors than did the controls. The malignant tumors were reticulum cell sarcomas, fibrosarcomas of the dermis, and squamous cell carcinomas of the skin, as well as one hepatocellular carcinoma in the liver. There was a metastatic fibrosarcoma in 2 rats ingesting Avadex, whereas no metastases were seen in the control rats.

BENIGN AND MALIGNANT TUMORS IN MALE RATS INGESTING AVADEX a/

Dose (ppm)	Incidence of Benign Tumors	Incidence of Malignant Tumors	Incidence of Total Tumors b/
0	2/50 (4%)	4/50 (8%)	5/50 (10%)
50	1/50 (2%)	7/50 (14%)	8/50 (16%)
100	4/49 (8%)	4/49 (8%)	8/49 (16%)
200	3/50 (6%)	7/50 (14%)	10/50 (20%)

a/ Number of rats with endocrine tumors are not included.
b/ Rats with both benign and malignant tumors were counted once.

CARCINOMAS AND SARCOMAS IN MALE RATS INGESTING AVADEX a/

Dose (ppm)	Incidence of Carcinomas	Incidence of Sarcomas	Incidence of Malignant Tumors b/
0	0/50 (0%)	4/50 (8%)	4/50 (8%)
50	3/50 (6%)	4/50 (8%)	7/50 (14%)
100	1/49 (2%)	3/49 (6%) c/	4/49 (8%)
200	2/50 (4%)	5/50 (10%)	7/50 (14%)

a/ Number of rats with endocrine tumors are not included.
b/ Rats with both benign and malignant tumors were counted once.
c/ One rat had metastatic fibrosarcoma to the lung and liver, another to the lung. No primary sarcoma was found in the two rats.

The incidence of benign and malignant tumors in untreated control female mice was so high that differences between them and the treated rats were not observed. One female rat ingesting 200 ppm Avadex had a fibrosarcoma of the dermis with metastases in multiple organs. One rat ingesting 50 ppm had metastases in the lung, without a known primary sarcoma, apparently from the dermis. Metastases did not occur except from mammary gland carcinomas in one control rat. Hemangiosarcoma of the liver was seen in another female given 200 ppm Avadex.

There were increased numbers of mammary gland tumors in female rats ingesting Avadex. There were mammary gland tumors in 29 of 49 rats (59%) given 100 ppm Avadex and in 19 of 50 control female rats (38%) ($p < .05$). The increase in carcinomas of the mammary gland was dose related. Nine of 46 female rats (20%) given the highest dose developed carcinomas compared to 4 of 50 female control rats (8%) ($p < .1$). It is likely that some, if not all, of the fibrosarcomas of the dermis were tumors of the mammary gland. These tumors would have further increased malignant tumors in that organ.

BENIGN AND MALIGNANT TUMORS OF THE MAMMARY GLAND IN FEMALE RATS INGESTING AVADEX a/

Dose (ppm)	Incidence of Benign Tumors	Incidence of Malignant Tumors	Incidence of Combined Tumors
0	15/50(30%)	4/50(8%)	19/50(38%)
50	19/49(39%)	4/49(8%)	23/49(47%)
100	24/48(50%)	5/48(10%)	29/48(60%)
200	15/46(33%)	9/46(20%)	24/46(52%)

a/ Some rats had both benign and malignant tumors. Those rats are counted once.

Pituitary tumors were also increased in rats ingesting Avadex. Treated female rats given Avadex has around a 50% incidence and female controls 33%. Some of the pituitary adenomas in treated female and male rats were probably carcinomas, as was found with tumors of the mammary gland. The tumors of the pituitary will be examined by CAG.

BENIGN AND MALIGNANT TUMORS OF THE PITUITARY GLAND
IN FEMALE RATS INGESTING AVADEX

Dose (ppm)	Incidence of Adenomas	Incidence of Carcinomas	Incidence of Tumors
0	16/45(36%)	0/45(0%)	16/45(36%)
50	22/41(54%)	0/41(0%)	22/41(54%)
100	15/39(38%)	0/39(0%)	15/39(38%)
200	15/32(47%)	0/32(0%)	15/32(47%)

BENIGN AND MALIGNANT TUMORS OF THE PITUITARY
GLAND IN MALE RATS INGESTING AVADEX

Dose (ppm)	Incidence of Adenomas	Incidence of Carcinomas	Incidence of Tumors
0	4/32(13%)	0/32(0%)	4/32(13%)
50	7/30(23%)	0/32(0%)	7/30(23%)
100	10/34(29%)	0/34(0%)	10/34(29%)
200	6/30(20%)	0/30(0%)	6/30(20%)

In this study, there were increases in tumors, particularly malignant tumors in male rats ingesting Avadex. Benign, as well as malignant, tumors of the mammary gland, occurred somewhat oftener in treated female rats. There also were some increases in adenomas of the pituitary gland in both male and female rats given Avadex. Control rats, particularly female, developed a high incidence of tumors, making it difficult to interpret the results. Histological examination of tissues in this study was limited.

In this study, female rats ingesting 100 ppm Avadex had more carcinomas of the mammary gland than did the controls. Otherwise, the high incidence of tumors that was seen in control rats make interpretation of the results difficult. Histological examination of tissues in this study was deficient.

V. SUMMARY

Male mice of two strains ingesting Avadex developed a significant increase in tumors in one or more organ as well as a significant increase in "hepatomas." Male mice of one strain ingesting Avadex also had more lung tumors than did the control mice.

There was a slightly higher incidence of mammary gland and uterine tumors in female mice given intraperitoneal injections or dermal applications of Avadex than in controls. The tumors also were more malignant in the Avadex-treated mice than in the untreated mice. There was also a slight increase in the incidence of tumors of the liver.

Male and female rats injesting Avadex developed significantly higher incidence of malignant tumors, than control rats. There was a dose-related increase in carcinomas in treated male rats. In another study, female rats had more carcinomas of the mammary gland than did the untreated rats.

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